PEROXISOMAL DISORDERS: A REVIEW ON CEREBELLAR PATHOLOGIES

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ABSTRACT

Peroxisomes are organelles with diverse metabolic tasks including essential roles in lipid metabolism. They are of utmost importance for the normal functioning of the nervous system as most peroxisomal disorders are accompanied with neurological symptoms. Remarkably, the cerebellum exquisitely depends on intact peroxisomal function both during development and adulthood. In this review we cover all aspects of cerebellar pathology that were reported in peroxisome biogenesis disorders and in diseases due to dysfunction of the peroxisomal α-oxidation, β-oxidation or ether lipid synthesis pathways. We also discuss the phenotypes of mouse models in which cerebellar pathologies were recapitulated and search for connections with the metabolic abnormalities. It becomes increasingly clear that besides the most severe forms of peroxisome dysfunction that are associated with developmental cerebellar defects, milder impairments can give rise to ataxia later in life.

INTRODUCTION

Peroxisomal function and dysfunction

Peroxisomes are omnipresent in mammalian cells but they have a flexible abundance and content depending on the cell type, developmental and physiological state. Beside peroxide generating oxidases and peroxide degrading enzymes such as catalase, these organelles harbor several pathways of lipid metabolism (140, 144, 148). Via α-oxidation they chain shorten the branched-chain fatty acid phytanic acid taken up via the diet and hydroxy-fatty acids that are enriched in myelin. Peroxisomal β-oxidation is not only essential for the degradation of very long chain fatty acids (VLCFA) and for the further breakdown of branched-chain fatty acids, but also for the synthesis of polyunsaturated fatty acids (PUFA) such as docosahexaenoic acid (DHA, C22:6ω-3) and the conversion of cholesterol into bile acids. Moreover, peroxisomes are required for the synthesis of ether lipids, including plasmalogens that are abundantly present in myelin. In addition, they take part in purine, polyamine, glyoxylate and amino acid metabolism. The ongoing determination of the peroxisomal proteome (59) is unveiling new peroxisomal proteins which might lead to the identification of new peroxisomal disorders.

Peroxisomal diseases can be categorized in biogenesis disorders (PBD) and single enzyme or transporter defects. PBDs arise when one of 14 *PEX* genes encoding so-called peroxins are defective (15). All these proteins are involved in a chain of events that is necessary to import cytosolically synthesized proteins into the peroxisomal membrane or lumen, or for peroxisome fission (151). Peroxisomal proteins that are mislocalized to the cytosol are mostly instable or inactive. Depending on the severity of the *PEX* mutation, this causes an array of disorders with developmental and/or degenerative pathology currently known as the Zellweger spectrum (15, 125).

Other peroxisomal disorders with neurological pathologies are due to mutations in peroxisomal enzymes or membrane transporters of the above mentioned lipid pathways (149). Although their metabolic deficits are less wide-ranging, the pathologies also vary from lethality in the postnatal period to isolated neurological demise in adulthood, depending on the affected gene and residual protein function.

We first discuss the cerebellar pathologies in single enzyme diseases as these can be better correlated with the metabolic dysfunction before elaborating on the PBD. The findings in patients and in the corresponding mouse models are dealt with in parallel.

Cerebellar development and architecture

The cerebellum is involved in the coordination of smooth, purposeful motor activities and in learning new motor skills. Moreover, a growing body of evidence infers additional cerebellar functions such as cognitive processing (e.g. language) and emotional reactivity (e.g. fear)(115).

In humans, cerebellar development is a slow process, starting in the embryonic stage and finishing in the first postnatal years whereas in mice it occurs postnatally over a three-week period. In newborn pups, Purkinje cells (PCs) extend multiple, non-organized dendrites evolving in a monolayer with primary dendrites branching in a single, parasagittal plane by postnatal day 21 (P21). By providing the only projections to the deep cerebellar nuclei (DCN) and vestibular nuclei, PCs are the sole output neurons of the cerebellar cortex (Figure 1). During the same timeframe granule cells migrate from the external granule cell layer (EGL) to the internal granule cell layer (IGL) and extend their parallel fibers (PF). This process of cell migration is carefully guided by Bergmann glia fibers

projecting into the molecular layer. After arrival in the IGL, granule cells mature and synaptic contacts are established.

Mature PCs are unique neurons with an elaborate dendritic tree harboring many spines, allowing a multitude of synaptic contacts. It has been shown that extrinsic signals such as normal synaptic input, growth factors, hormones and neurotrophins are necessary for dendritic outgrowth and refinement. Initially, each PC receives excitatory input from multiple climbing fibers (CF), originating from the contralateral inferior olivary nucleus of the medulla. By P20 surplus CF are eliminated, resulting in mono innervation. This CF – PC connection is one of the strongest in the central nervous system (CNS). Indirect PC innervation originates from axons arising from the spinal cord and brainstem. These so-called 'mossy fibers' project to the granule cells, which in turn send PF making synaptic contacts with dendritic spines of multiple PCs. Excitatory inputs on PCs are counteracted by basket- and stellate cells, both inhibitory interneurons present in the molecular layer. The circuit is closed by inhibitory feedback connections from the DCN to the inferior olivary nucleus and an excitatory feedback projection between the cerebellar nuclei and the granule cells (Figure 1). Moreover, neuronal connections between the cerebellum and diverse brain regions such as the basal ganglia, hippocampus, thalamus and hypothalamus should not be overlooked. Given this extensive cerebellar wiring, the aforementioned additional roles in higher order brain functions are not surprising.

Pathologies causing abnormalities in PC development, cerebellar malfunctioning or PC loss generally cause a range of motor disturbances (e.g. tremor, dysmetria and hypotonia) collectively referred to as 'cerebellar ataxia'. Currently, more than 60 different types of cerebellum-based ataxias have been identified. In what follows, cerebellar anomalies caused by peroxisomal dysfunction during development and adulthood will be discussed in men and mice.

DEFECTS IN α-OXIDATION OF PHYTANIC ACID

Patients lacking the first enzyme of the peroxisomal α-oxidation pathway, phytanoyl-CoA hydroxylase (PHYH), suffer from Refsum disease, an inherited disorder resulting in the accumulation of phytanic acid in several tissues and body fluids (146, 153). Although the majority of Refsum patients carry *PHYH* gene mutations, the disease can also be caused by *PEX7* mutations, impairing the import of PHYH into the peroxisome (64, 67, 68, 136) (Figure 2). Patients lacking other enzymes of the peroxisomal α-oxidation pathway have not been identified. Phytanic acid is derived from chlorophyll by bacterial metabolism in the gut of ruminants and is primarily taken up through ruminant meat and dairy products. Other peroxisome-related disorders characterized by increased phytanic acid levels are Zellweger spectrum disorders, multifunctional protein 2 (MFP2) deficiency, α-methylacyl-CoA racemase deficiency and rhizomelic chondrodysplasia punctata (RCDP) type 1.

Refsum disease

Refsum was the first to describe cerebellar signs as one of the main manifestations of the disease that was named after him, although it is now clear that cerebellar problems can be late in onset compared to retino- or neuropathy (145). The clinical presentation of Refsum disease is variable, and cerebellar signs do not always occur (3, 121). Surprisingly, only few postmortem histopathological investigations on Refsum patients were performed and

findings in the cerebellum and brainstem are scarce, revealing atrophy of the cerebellar vermis and patchy cell degeneration in the dentate and inferior olivary nuclei (22, 56, 107). It should be noted that cerebellar pathology may not be the sole origin of ataxia as gait disturbances without cerebellar pathological changes have been reported (46, 113). The loss of proprioception caused by the demyelinating neuropathy will cause a sensory ataxia on top of the cerebellar ataxia (46, 89). As their gait disturbances ameliorated upon dietary phytanic acid restrictions, this strongly indicated that phytanic acid accumulation induces toxicity (33). Interestingly, phytanic acid concentrations are 3 to 4 times higher in the peripheral nerves of Refsum patients as compared to the brain, due to impaired access through the blood-brain barrier (131). Phytanic acid accumulations in the peripheral nervous system slow down nerve conduction velocity, impair reflexes and perturb sensation. The demyelinating polyneuropathy generally starts before the cerebellar dysfunction, making it sometimes clinically difficult to detect the cerebellar ataxia. However, signs like nystagmus cannot be explained by the polyneuropathy, and other signs of ataxia are out of proportion with the degree of sensory involvement (134).

The Refsum mouse model

The *in vivo* short-term pathological processes of Refsum disease were studied by Ferdinandusse *et al.* (44), using PHYH deficient mutant mice on chow supplemented with phytol. Phytanic acid is derived from dietary sources only and the concentration of branched-chain fatty acids or their precursors in standard rodent food is low. A moderate increase in the plasma concentration of phytanic acid was obtained when *Phyh*$^{-/-}$ mice were supplemented with 0.1% phytol for six weeks, whereas three weeks of 0.25% phytol-supplementation yielded plasma levels similar to those reported in Refsum disease patients (> 1 mmol/L). Phytanic acid markedly accumulated in the cerebellum, although levels were 3-10 fold lower compared to testis, kidney and liver, in line with the relative impermeability of the blood-brain barrier (131). The phytol diet induced prominent gait disturbances in the mutant mice. Immunohistochemistry revealed cerebellar modifications ranging from focal PC loss in *Phyh*$^{-/-}$ mice fed 0.1% phytol to severe cell loss and morphological changes of the remaining cells in *Phyh*$^{-/-}$ mutants on the 0.25% phytol-supplemented diet. As in patients, also in the phytol supplemented *Phyh*$^{-/-}$ mice, the peripheral nervous system contributed to the symptoms as motor nerve conduction velocity was decreased, indicating peripheral neuropathy.

Molecular aspects of phytanic acid accumulation

The improvement of ataxia following reduced phytanic acid intake in Refsum patients, as well as the dosage-dependent severity of PC degeneration in the phytol treated Refsum mouse model, strongly suggest a detrimental role of high phytanic acid concentrations on the cerebellum.

During the past decade several *in vitro* studies were performed to elucidate the mechanisms underlying the toxicity caused by phytanic acid. These include experiments with isolated cerebellar mitochondria, cerebellar homogenates, cultured neural cells or fibroblasts incubated with phytanic acid concentrations in the range of those found in plasma of Refsum disease patients (1 – 500 µM). Impairments of mitochondrial function and morphology were commonly found, including a reduced matrix NAD(P)H content, alterations in energy production, loss of membrane potential and opening of the transition pore (19, 69, 75, 109, 111, 116). Phytanic acid also induced oxidative stress in some *in vitro* preparations (109).

As to the precise molecular impact, opposing mechanisms were proposed, probably due to the varying experimental setups. In view of the low permeability of the blood-brain barrier to phytanic acid (131), it can be questioned whether exposure of neural cells, and in particular isolated mitochondria, to phytanic acid concentrations occurring in Refsum plasma reflects the *in vivo* situation. In some experiments, 80% of cultured glial cells died after a four hour incubation with 100 µM phytanic acid (111) whereas, with exception of PCs, no overt cell death was reported in the brain of Refsum mice treated with a high dose of phytol (44). In addition, it is unfortunate that in several studies control incubations with straight-chain fatty acids were not included. In a well-controlled study using permeabilized and intact human fibroblasts (75) it was shown that phytanic acid acts as a protonophore leading to mitochondrial membrane depolarization and reduced ATP production. It was further proven that this activity depended on the branched-chain and carboxyl function as it was mimicked by pristanic acid, the α-oxidized shortened product of phytanic acid, but not by phytol or the straight-chain palmitic acid with the same number of carbons in the backbone. In addition to mitochondrial dysfunction, it was reported that phytanic acid impairs Ca^{2+} homeostasis (69, 111) and deregulates synaptic Na^{+}, K^{+}-ATPase in the cerebellum (20). Taken together, phytanic acid seems to exert adverse effects on cerebellar PCs, which contribute to an unstable gait in men and mice. Nevertheless, the potential mechanisms deduced from *in vitro* experiments need to be confirmed in the *in vivo* situation, which is possible using the Refsum mouse model.

PEROXISOMAL β-OXIDATION DISORDERS

Currently, six divergent genetic diseases are known in which peroxisomal fatty acid β-oxidation is deficient. In order of decreasing incidence the following pathologies are distinguished: (1) X-linked adrenoleukodystrophy (X-ALD), (2) multifunctional protein-2 (MFP2) deficiency, (3) acyl-CoA oxidase 1 (ACOX1) deficiency, (4) 2-methylacyl-CoA racemase (AMACR) deficiency, (5) sterol carrier protein X (SCPx) deficiency and (6) ABCD3 deficiency (Figure 2).

X-linked adrenoleukodystrophy (X-ALD)

Adrenoleukodystrophy protein (ALDP), the peroxisomal ABC transporter encoded by the ATP binding cassette D1 (*ABCD1*) gene, transports VLCFA either as free acids or as CoA esters into peroxisomes prior to β-oxidation (27, 154). As a consequence, ALDP deficiency is biochemically characterized by $C_{24:0}$ and $C_{26:0}$ accumulations in plasma and tissues such as the CNS, adrenal cortex and gonads.

Cerebellar and brainstem pathologies in X-ALD patients

In X-ALD patients, the clinical spectrum ranges from asymptomatic and Addison-only (isolated adrenocortical insufficiency) to progressive neurological dysfunction due to cerebral demyelination in childhood, adolescence or adulthood (71).

The majority of X-ALD males present either with childhood cerebral ALD (CCALD), characterized by major inflammatory demyelination of the cerebral white matter, or with adrenomyeloneuropathy (AMN), a progressive disorder involving the spinal cord and peripheral nerves (with more frequent axonal than demyelinating polyneuropathy) in adulthood. In both patient groups, adrenocortical failure, which also occurs as the sole clinical involvement, is frequent. About half of AMN patients develop cerebral white matter lesions, typically with a less aggressive evolution than the lesions in CCALD. These different presentations can occur in the same family (with

the same ABCD1 mutation), and the factors determining who will develop which clinical presentation remain largely unknown. Moreover, recent evidence indicates that bone marrow transplantation, the therapy for CCALD, is not effective to prevent AMN, again indicating the differences in pathogenesis of these presentations (138). In about 1% of patients (86), the white matter affection of CCALD and AMN starts in the cerebellar white matter, resulting in cerebellar ataxia as neurological presentation in these patients. Also, a form of X-ALD presenting with primary cerebellar (neuronal) degeneration has been described (80). Almost all of these latter cases were reported before genetic confirmation of X-ALD was part of clinical routine, and before the mild presentations of peroxisomal biogenesis disorders (138) (among which ataxia) were known. As the finding of elevated VLCFA was the only biochemical proof of the diagnosis, several of these cases may in fact have been patients with a mild PBD instead of X-ALD. At least one report describes profound vermian atrophy in a genetically confirmed X-ALD patient, even without white matter changes. The family history was suggestive of a cerebellar presentation in other affected family members, and the authors proposed that this presentation was linked to this particular mutation, delC1321in exon 2 (31). Reports of more confirmed patients with this presentation would help to establish its real frequency.

Insights from mouse models

Three *Abcd1* knockout mouse models were generated independently (48, 71, 73, 88), all showing VLCFA accumulation in the brain. A late-onset phenotype affecting motor performance developed in 20-month-old *Abcd1*$^{-/-}$ mice and was accompanied by axonal degeneration in the spinal cord, resembling AMN pathology. Remarkably, inflammatory demyelination as seen in CCALD patients did not develop in the CNS (105). However, PC death was reported in the cerebellum of aged ABCD1 deficient mice (45), corresponding with the rare X-ALD patients with primary cerebellar atrophy.

ABCD2 deficiency, so far not described in men, was created in mice. This transporter partially overlaps in function with ABCD1 (93). Similar to *Abcd1* mutants, ABCD2 deficient mice developed cerebellar problems including PC atrophy and loss, concomitant with whole body tremor and cerebellar ataxia. Not surprisingly, the pathological signs occurred earlier and were more severe in *Abcd1*/*Abcd2* double mutants (45).

The toxicity of VLCFA

The only metabolic abnormality in X-ALD is the accumulation of VLCFA. Because of their exceptional length, increased levels of VLCFA are thought to be detrimental when incorporated into membrane phospholipids (62). The toxicity of $C_{26:0}$ was shown in several *in vitro* studies using diverse cell types including neural cells. In some experimental designs, tissues or cells with *Abcd1* gene knockout or knockdown were used giving rise to elevated VLCFA levels from endogenous origin (4, 53). In other studies, cells were incubated with exogenously added $C_{26:0}$ up to 50 µM (61, 87, 155). It should however be noted that this exceeds the pathological concentrations of $C_{26:0}$ in X-ALD patients which were estimated to be 1-5 µM (5, 28, 129, 135). Oxidative stress and mitochondrial impairments were recurrent cellular dysfunctions triggered by the VLCFA and were assumed to cause axonal degeneration in the spinal cord (52) and, together with an inflammatory component, the cerebral phenotype (120). Whether these mechanisms also underlie the demise of PCs is however unresolved.

Acyl-CoA Oxidase-1 (ACOX1) deficiency

ACOX1 catalyzes the first step in peroxisomal β-oxidation. Its enzymatic function is restricted to the oxidation of VLCFA ($\geq C_{24:0}$), long-chain dicarboxylic acids and PUFA. ACOX1 deficiency is often referred to as pseudo neonatal ALD (pseudo-NALD) because of the similarities in clinical presentation with the peroxisome biogenesis disorder NALD (see below) (101). Patients have increased levels of VLCFA (39) although some ACOX1 deficient patients with normal plasma VLCFA concentrations have also been described (112).

Currently, the pathogenic knowledge of ACOX1 deficiency is restricted to what has been observed in a limited number of patients worldwide. Delays in motor development such as head control, crawling and non-assisted walking were described. In addition to a delay in attaining early developmental milestones, almost all ACOX1 deficient children aged 24 to 48 months showed regression of motor achievements. Brain MRI studies demonstrated myelin loss starting in the white matter of the cerebellum, cerebellar peduncles and brainstem tracts, progressively extending to the midbrain and the cortical white matter (21, 81, 101, 112, 126, 147, 150).

In two ACOX1 deficient siblings with a mild disease course, developmental milestones were normal but clumsiness appeared at an age of 8 - 10 years evolving in progressive unsteadiness and severe ataxia. Interestingly, the cerebellum, middle cerebellar peduncles and brainstem showed marked atrophy whereas the cerebrum was only mildly affected (38). These patients survived into their fifties. This moderate clinical phenotype was associated with only borderline increased levels of VLCFA, likely due to a mutation outside of the catalytically active parts of the enzyme (51, 126).

Acox1$^{-/-}$ mice present with a severe hepatic phenotype and, to our knowledge, the neuropathology has not been thoroughly investigated. Given that VLCFA accumulate in blood and ACOX1 expression was confirmed in neural and glial cells of the cerebellum and other motor-related regions such as the pontine nuclei and inferior olive (35, 49) it is surprising that no neurological signs were reported.

Multifunctional protein-2 deficiency

Multifunctional protein 2 (MFP2) deficiency occurs with an incidence of approximately 1:100.000 (41). MFP2, alternately known as D-bifunctional protein (DBP), is encoded by the hydroxy-steroid dehydrogenase type 4 (*HSD17B4*) gene. MFP2 catalyzes the oxidation of a broad spectrum of substrates including VLCFA, 2-methyl-branched-chain fatty acids (e.g. pristanic acid), bile acid intermediates (dihydroxycholestanoic acid (DHCA) and trihydroxycholestanoic acid (THCA)), leukotrienes and long-chain dicarboxylic acids. In addition, MFP2 is involved in the biosynthesis of docosahexaenoic acid (DHA; $C_{22:6n-3}$), the most abundant polyunsaturated fatty acid (PUFA) in the mammalian brain and retina (41).

Cerebellar and brainstem pathologies in MFP2-deficient patients

The importance of MFP2 in the developing CNS is highlighted by the fact that the majority of MFP2 deficient patients display severe abnormalities at birth, presenting with hypotonia and brain malformations, and die within the first years of life (41, 149). This phenotype is indistinguishable from Zellweger disease (see below). Concerning the cerebellum, imaging experiments uncovered hypoplasia, demyelination and atrophy. Brain autopsy studies revealed ectopic or degenerating PCs, gliosis and defects in the migration and maturation of granule neurons. Brainstem pathologies often include demyelination and malformations of the inferior olivary and dentate nuclei (41, 70, 72, 96, 99, 139).

Depending on the nature of the *HSD17B4* mutation, MFP2 deficient patients may achieve some developmental milestones followed by regression. Similar to mild PBD patients (see below) central cerebellar demyelination is then a common feature (41, 123). Importantly, several patients who presented with progressive cerebellar ataxia during childhood, adolescence or adulthood combined with peripheral neuropathy and hearing loss were identified in recent years as *HSD17B4* mutants by next generation sequencing (83, 85, 90). The residual enzyme activity led to normal plasma levels of VLCFA and branched chain fatty acids and they were proposed to represent a novel subtype of MFP2 deficiency based on their clinical, genetic and biochemical features.

Of interest, several syndromes encompassing cerebellar manifestations could also be ascribed to MFP2 deficiency. For example, *HSD17B4* mutations are the first recognized genetic cause of Perrault syndrome (100), characterized by ovarian dysgenesis and sensorineural hearing loss but often also associated with neurological problems. Shrinkage of the cerebellum and motor problems in Perrault patients are similar to the clinical profile of mild MFP2 deficient patients (47, 58, 97, 100). However, mutations in several other genes have been identified to cause Perrault syndrome. Furthermore, the importance of MFP2 in ataxic phenotypes can be deduced from patients with Stiff-man syndrome, a rare disorder of the CNS characterized by the production of auto-antibodies against glutamic acid decarboxylase, resulting in muscle rigidity and spasms. MFP2 is an autoimmune target in a subset of Stiff-man patients (26, 30, 63) and gait ataxia is a facultative neurological symptom (76). A correlation between the concentration of MFP2 auto-antibodies and the severity of cerebellar ataxia has not been established so far, but the expression of both GAD and MFP2 (98) in cerebellar PCs is in favor of PC dysfunction underlying the motor problems in these patients. Another post-developmental disease linked to MFP2 deficiency is optico-cochleo-dentate degeneration (OCDD) characterized by cerebellar symptoms, regression of motor performance and degeneration of the optic nerve, the dentate nucleus and the cochlear nerve (117). Clinically, the distinction between mild MFP2 deficiency, Perrault syndrome caused by MFP2 deficiency, and OCDD appears artificial, and all these syndromes could be gathered under "MFP2 ataxia syndrome".

Cerebellar and brainstem abnormalities in mouse models with MFP2-deficiency

In contrast to patients with a total ablation of MFP2 function, cerebral development was normal and cerebellar formation was only mildly affected in *Mfp2*$^{-/-}$ mice (6, 66). A delay in cerebellar foliation was observed in seven days old *Mfp2*$^{-/-}$ mice, but cerebellar architecture appeared normal in the post weaning period (77). However, progressive motor problems such as clasping of hind legs and poor performance on the rotarod already presented in four weeks old *Mfp2*$^{-/-}$ mice and evolved in immobility and death by the age of six months (66, 141). Peripheral sensorimotor abnormalities were absent in *Mfp2*$^{-/-}$ mice, pointing to MFP2 deficiency in the CNS as the origin of the motor problems (66). Studies in *Nestin-Mfp2*$^{-/-}$ mice, a model in which MFP2 is deleted from neural cells, showed a similar motor phenotype as the *Mfp2*$^{-/-}$ mice (141).

Histologically, several lesions were found in the cerebellum of both mouse models. Prominent lipid droplets containing neutral lipids progressively accumulated in Bergmann glial cells, whereas smaller inclusions were occasionally observed in cerebellar PCs. Interestingly, astrogliosis and up-regulation of the anti-oxidant enzyme catalase were also found in the molecular layer of the *Mfp2*$^{-/-}$ cerebellum (66, 141), similar to what has been observed in patients with defects in peroxisomal β-oxidation (54). These observations raised the question whether astroglial lesions underlie the neuromotor problems in MFP2 deficiency. It is however unlikely that the lipid droplets and catalase overexpression are detrimental as they also occurred in the brain of *Gfap-Pex5*$^{-/-}$ mice (see

also below) that do not develop neurological problems and have a normal life span. Additional microscopic analyses in both *Mfp2*$^{-/-}$ and *Nestin-Mfp2*$^{-/-}$ mice uncovered swellings on myelinated PC axons, followed by cerebellar microgliosis and profound astrogliosis in the deep cerebellar nuclei (DCN). Myelin loss started in the cerebellar lobules and expanded to the central white matter. In 12 months old *Nestin-Mfp2*$^{-/-}$ mice, a time point at which *Mfp2*$^{-/-}$ mice already passed away, degeneration of the PC axon and dendritic tree culminated in PC loss and cerebellar atrophy. The fact that axonal abnormalities preceded neurodegeneration was compatible with a dying back neuropathy (141). It was investigated whether absence of oligodendroglial MFP2 plays a causal role in the cerebellar demyelination by using *Cnp-Mfp2*$^{-/-}$ mice, an oligodendrocyte selective knockout model. However, these mice showed normal motor skills and their cerebellum was unaffected until the age of 12 months ruling out a primary role for peroxisomal β-oxidation in oligodendrocytes for the maintenance of the cerebellum (141).

The early-onset ataxic phenotype of both *Mfp2*$^{-/-}$ and *Nestin-Mfp2*$^{-/-}$ mice clearly preceded cerebellar histopathological changes (92, 141). As in other neurodegenerative diseases such as Huntington's disease (133) and spinocerebellar ataxia type 1 (65) early cell dysfunction rather than cell death might be involved in the initial disease stages. Currently, the cellular mechanisms underlying loss of PC integrity and cerebellar demise in MFP2 knockout mice are still unsolved. One of the outstanding questions is whether the PC degeneration is inherent to peroxisomal β-oxidation loss within these cells, which is supported by their elevated MFP2 expression as compared to other neural cells (92), or by non-cell autonomous mechanisms.

Candidate metabolites causing cerebellar defects in MFP2 deficiency

Given the broad substrate spectrum of MFP2, several metabolic disturbances could account for cerebellar degeneration in MFP2 deficient patients and mice. However, it remains unresolved how defective peroxisomal β-oxidation impacts on cerebellar integrity. First, as in ABCD1 deficiency, VLCFA accumulate in the nervous system after loss of MFP2. However, when comparing the mouse models, it is striking that cerebellar degeneration occurs much earlier and is more drastic in *Mfp2*$^{-/-}$ than in double *Abcd1/Abcd2*$^{-/-}$ mutants. Furthermore, the absence of a clinical phenotype in *Cnp-Mfp2*$^{-/-}$ mice, despite elevated $C_{26:0}$ levels in the cerebellum, does not support a crucial role for VLCFA in cerebellar degeneration (141).

MFP2 is also necessary for the breakdown of pristanic acid, the branched-chain fatty acid formed by α-oxidation of phytanic acid, both presumably having a similar toxicity profile when accumulating. As already mentioned, mice are exposed to very low levels of these fatty acids when fed a normal diet. Whereas in the Refsum mouse model the cerebellum only degenerated after supplementation of phytol, this occurred in MFP2 deficient mice on a standard diet. In addition, the cerebellum of *Mfp2*$^{-/-}$ and *Nestin-Mfp2*$^{-/-}$ mice is free of phytanic or pristanic acid accumulation and the ataxic behavior of *Mfp2*$^{-/-}$ mice fed with pellets enriched in the branched-chain fatty acid precursor phytol does not differ from MFP2 knockout mice on a normal diet (66). As already mentioned, it is striking that plasma levels of VLCFA and the branched-chain fatty acids are normal or near-normal in MFP2 patients with post developmental cerebellar degeneration (MFP2 ataxia syndrome) (38, 83, 85, 90, 100, 123).

Another potential metabolic candidate is a lack of DHA (C22:6ω-3). It is well established that this PUFA requires one peroxisomal β-oxidation cycle for its synthesis and in MFP2 deficient patients a shortage of this important PUFA was shown (40). Quite surprisingly, and for unclear reasons, DHA levels are normal in cerebellum and other brain regions of MFP2 knockout mice (66). Unless cell type or regional differences in DHA concentration would exist, a lack of PUFA cannot be claimed as the origin of cerebellar pathology.

In summary, the cerebellar pathology in *Mfp2*$^{-/-}$ and *Nestin-Mfp2*$^{-/-}$ mice cannot be correlated with levels of known MFP2 substrates nor by myelin loss. It appears therefore that MFP2 could have other, unknown functions.

Alpha-Methylacyl-CoA Racemase (AMACR), Sterol Carrier Protein X (SCPx) and ABCD3 gene mutations

Alpha-Methylacyl-CoA Racemase (AMACR), SCPX and PMP70 are involved in the metabolism of branched chain substrates by peroxisomal β-oxidation. AMACR converts 2R-pristanic acid, 25R-DHCA and 25R-THCA to their S-configuration, prior to their passage through the peroxisomal β-oxidation pathway. Most AMACR deficient patients are asymptomatic until adolescence whereafter they start showing symptoms similar to those observed in Refsum disease patients (29, 60, 132). Cerebellar dysfunction was only seen in two out of ten patients (24, 29, 60). An MRI study on these two siblings revealed chronic degenerative lesions of efferent cerebellar pathways (60). Altered signal intensities in the dentate nucleus, superior cerebellar peduncle, thalamus and red nucleus indicated degeneration of the cerebellothalamic, dentatothalamic and dentatorubral tracts. In addition, pontine atrophy and signal changes of the transverse pontine fibers also revealed involvement of cerebellar afferents. No inferior olivary lesions were observed (60). The sensory (-motor) neuropathy observed in most patients likely contributes to their motor abnormalities (60).

Pristanic acid also accumulates in patients with mutations in the *Sterol Carrier Protein X* (*SCPx*) gene. Once SCPx is imported into the peroxisome, it is split into two functional domains: a thiolase domain that catalyzes the final step of the peroxisomal β-oxidation, and a sterol carrier protein 2 (SCP2) domain. Gait disturbances, tremor and impairment of balance in an adult SCPx deficient patient was consistent with cerebellar ataxia and slowed saccades indicated brainstem malfunction (43). As in other peroxisomal patients with defective branched-chain fatty acid catabolism, the motor and sensory neuropathy should not be overlooked as an additional source of motor problems. Similar to Refsum disease patients, restriction of phytanic acid intake halted symptom progression in the SCPx patient (38, 43), while in AMACR deficient patients the benefit of a phytanic acid restricted diet is inconclusive (122). *Scpx* knockout mice challenged with a phytol diet developed ataxia and peripheral neuropathy with an unsteady gait (118) but this was not the case in *Amacr* knockouts (114). Unfortunately, the cerebellum was not investigated in these mouse models.

Finally, the transport of the C_{27}-bile acid intermediates and branched chain fatty acids across the peroxisomal membrane requires ABCD3 also denoted as PMP70. In a single recently identified ABCD3 deficient patient and in the mouse model no cerebellar defects were observed (42). However, the patient died at young age from liver disease and the mice were only supplemented with phytol for a short period, precluding potential effects of branched chain fatty acids on the central or peripheral nervous system.

ETHER PHOSPHOLIPID DEFICIENCY

Ether phospholipids are a subclass of glycerophospholipids with a long chain fatty alcohol at the sn-1 position. Of all ether lipid species, plasmalogens that have a vinyl-ether bond are the most abundant in mammalian tissues (16, 82, 95). The synthesis of this ether bond requires the enzymatic machinery in peroxisomes, namely glyceronephosphate O-acyltransferase (GNPAT) and alkylglycerone phosphate synthase (AGPS) (57). Being an essential constituent of myelin and neurological membranes, plasmalogens are thought to have a pivotal role in

signal transduction both in the central – and peripheral nervous system. At the molecular level, they were suggested to act as antioxidants, as a reservoir of PUFA and as mediators of membrane dynamics (16, 83, 95).

Rhizomelic Chondrodysplasia Punctata (RCDP)

Rhizomelic Chrondrodysplasia Punctata (RCDP) has an overall incidence of 1:100.000 (9). RCDP type 1 is caused by mutations in the PTS2 receptor PEX7, impairing the import of three peroxisomal enzymes i.e. AGPS, phytanoyl-CoA hydroxylase and 3-ketoacyl thiolase. The majority of PEX7 deficient patients show a very severe to moderate depletion in plasmalogen levels together with an increase in plasma phytanic acid levels (9). In a few *PEX7* patients (64, 136) mutations are so mild that plasmalogen levels are close to normal and, as already mentioned, their phenotype is similar to Refsum disease patients, due to impaired activity of phytanoyl-CoA hydroxylase (68). In all other cases the phenotype is fully determined by the impaired ether phospholipid synthesis. This is deduced from the fact that the clinical signs are indistinguishable from patients with RCDP type 2 and 3 with an isolated defect in ether-phospholipid synthesis caused by mutations in respectively *GNPAT* and *AGPS*.

Cerebellar and brainstem pathologies in RCDP patients

The clinical image of RCDP types 1, 2 and 3 is generally severe but varies according to the residual capacity in plasmalogen biosynthesis (10). The most severely affected patients with undetectable plasmalogens in red blood cells showed progressive cerebellar atrophy due to PC and granule cell degeneration in the neonatal period. The onset of cerebellar deterioration was delayed by one year in patients with residual albeit very low plasmalogen levels (9, 10). Non-inflammatory myelin abnormalities were frequently observed (2, 104, 127, 128) and dysplastic olives and cerebellar heterotaxias noted in post-mortem studies may refer to developmental defects (102, 104, 124). The achievement of motor skills in patients with the severe phenotype is poor (10). Other typical features include skeletal anomalies, audiovisual problems and psychomotor retardation with a considerably reduced life expectancy.

Less is known about the disease process of chronic RCDP patients with a milder clinical and biochemical course. The majority shows a delay in motor development such as the inability to sit or walk independently (9, 10, 12). Furthermore, patients with mild ataxia, nystagmus and cerebellar atrophy with Purkinje- and granule cell depletion have also been reported (103). As in the severely affected children, abnormalities in cerebellar architecture or myelination were not always present in these mild RCDP patients (10, 12).

Insights from mouse models

Knowledge about the consequences of deficient ether lipid synthesis has greatly increased by the analysis of *Pex7*$^{-/-}$ and *Gnpat*$^{-/-}$ (also called *Dhapat*$^{-/-}$) mouse models (14, 17, 84, 110). Cerebellar development was mainly studied in *Gnpat* knockout mice revealing foliation abnormalities, a delay in granule cell migration and atrophy during the first postnatal weeks (77, 130). The PC showed multiple aberrations including axonal spheroids, defects in paranode organization and a hyperspiny appearance accompanied by altered CF and PF innervations (130). Dysmyelination occurred both in the cerebellum and the cerebrum (130) but cerebellar myelin loss was not progressive (13). Decreased rotarod and vertical pole performances in *Gnpat*$^{-/-}$ mice suggested cerebellar ataxia (130). However, a recent study on both *Pex7* and *Gnpat* knockout mice has shown that plasmalogen deficiency in the peripheral nervous system affects axonal sorting and the myelination process by influencing Schwann cell

differentiation and maturation, thereby causing peripheral neuropathy. Impairment of the AKT – GSK3β pathway induced by plasmalogen deficiency in Schwann cell membranes was proposed as the underlying mechanism (25). Therefore, the ataxia of *Gnpat*$^{-/-}$ mice may have a dual origin.

Fatty Acyl-CoA Reductase 1 (FAR1) deficiency

The importance of plasmalogens for the maintenance of cerebellar integrity is underscored by the recent identification of a few patients with FAR1 deficiency. FAR1 plays a critical role in the plasmalogen biosynthesis pathway by reducing saturated and unsaturated fatty acyl-CoAs to their respective fatty alcohols. The enzyme is located at the cytosolic side of the peroxisomal membrane. Remarkably, a 19 year old FAR1 deficient patient presented with atrophy of the cerebellar vermis and hemispheres accompanied by cortical white matter lesions (18).

PEROXISOME BIOGENESIS DEFECTS

The import of peroxisomal matrix and membrane proteins requires the concerted action of at least 14 peroxins that have diverse functions including cytosolic receptors (PEX5 and PEX7) for enzymes carrying the peroxisome targeting signal (PTS) 1 or 2, docking proteins in the peroxisomal membrane and proteins that allow the membrane translocation and the recycling of receptors to the cytosol (151). PEX7 is only needed for the import of a minority of proteins harboring a PTS2 signal (AGPS, phytanoyl-CoA hydroxylase and 3-ketoacyl thiolase). As already mentioned, severe PEX7 mutations cause RCDP whereas very mild mutations cause Refsum pathologies due to respectively plasmalogen synthesis and α-oxidation defects (136). Defects in all the other peroxins will impede all peroxisomal functions leading to an array of clinical presentations now designated as Zellweger spectrum disorders (15, 125). The phenotypes range from severe developmental disorders (Zellweger syndrome), that are fatal in the first year of life, to milder syndromes previously named Neonatal adrenoleukodystrophy and Infantile Refsum disease that sometimes allow survival into adulthood.

Developmental cerebellar pathologies

Newborns with Zellweger syndrome, also known as the cerebro-hepato-renal syndrome, present with hypotonia and craniofacial dysmorphisms (15). MRI of the brain shows gyric abnormalities including polymicrogyria in the Sylvian fissure, frontoparietal pachygyria and heterotopias (11, 152), indicative of a neuronal migration defect. Brain atrophy is another common feature. The landmark histological studies by Volpe (142) and Evrard (34) showed that in Zellweger syndrome besides the cytoarchitectonic abnormalities of the cortex, also malformations of the cerebellum and the inferior olivary nucleus occur. In the postnatal cerebellum, a subset of PCs are heterotopically located in the white matter, or abnormally positioned relative to the granule cells as a result of hampered neuronal migration. The dysplasia of the olivary and dentate nuclei also encompass laminar discontinuities. By MRI, no abnormalities were however observed in the cerebellum within the age of 2 months (11).

Cerebellar malformation in PBD was studied in detail using mouse models lacking PEX2, PEX5 or PEX13. Depending on the genetic background, the global knockout mice die in the perinatal period or survive only for a few weeks (7). Only in the longer surviving *Pex2*$^{-/-}$ knockouts, cerebellar development could be studied as it occurs

postnatally in mice. Alternatively, neural selective knockouts were generated for PEX5 and PEX13 circumventing severe liver pathology in the postnatal period and enabling to study cerebellar histogenesis (78, 94).

A thorough investigation in *Pex2*$^{-/-}$ mice revealed multiple anomalies in the developing cerebellum in which granule cells, PC and climbing fibers were affected leading to persistent abnormalities in cerebellar foliation (36). The migration of granule cells from the EGL to the IGL was delayed. As the Bergmann glia scaffold appeared normal the migration impairment was due to an intrinsic problem of the granule cells which also showed increased apoptotic death. The impaired granule cell migratory capacity was confirmed using *in vitro* setups (37). The heterotopia of PCs was less pronounced in the mice as compared to Zellweger patients as only a minor fraction of PCs were slightly delocalized to the IGL. However, although the initial development of PC was indistinguishable from wild type until postnatal day 3, the subsequent maturation was impaired with less complex branching of the dendritic tree and aberrant formation and orientation of dendritic spines. The latter coincided with the abnormal development of climbing fibers. Although these innervated the PC somata at P3 in a normal fashion, the translocation of the climbing fiber dendritic tree to the PC dendrite compartment was delayed (36). In addition, also the axonal compartment of PCs was affected with the occurrence of spheroids, altogether indicative of ongoing degenerative processes.

These data were confirmed in mice with inactivation of *Pex13* (94) or *Pex5* (78) restricted to neural cells by using *Nestin-Cre* and respective floxed mice. Their cerebellar development was abnormal including granule cell migration impairment, increased cell death and defects in PC positioning and arborization. In cultured cerebellar *Pex13*$^{-/-}$ neurons, oxidative stress mediated by mitochondrial dysfunction was demonstrated (94). Surprisingly, no elevation of VLCFA was found in *Nestin-Pex13*$^{-/-}$ brains eliminating this as a potential mechanism.

Remarkably, also in mice with a liver selective depletion of functional peroxisomes, major abnormalities in cerebellar morphogenesis leading to impaired foliation were observed (78), indicating that brain extrinsic mechanisms also play a role. The mechanisms were not resolved but given that bile acid treatment can partially restore the cerebellar anomalies in *Pex2* knockout mice, accumulation of immature bile acids may be involved (37).

Taken together, the conservation of cerebellar dysgenesis in peroxisome deficient mice and men, underscores the necessity of intact peroxisomal metabolism for the normal formation of the cerebellum. Both nervous system intrinsic and extrinsic factors may impact on the intricate interplay between granule cells, climbing fibers and PCs.

Milder PBD and their cerebellar pathologies

The milder Zellweger spectrum disorders have a variable onset and presentation. The age at diagnosis ranges from the neonatal period up to adulthood and survival varies from a few years to several decades. Impairment of vision and hearing, psychomotor retardation and liver disease are among the most frequent clinical signs. Other symptoms include hypotonia, leukodystrophy and ataxia. A major obstacle for the diagnosis is that the typical peroxisome related biochemical anomalies (increased levels of VLCFA, branched-chain fatty acids, bile acid intermediates and plasmalogens) are often not or only borderline affected. Frequently, the genetic cause is a point mutation in *PEX1*, the gene accounting for more than 50% of Zellweger spectrum cases (151).

Leukodystrophies initiate in the hindbrain

Infants with the adrenoleukodystrophy phenotype of PBD reach some developmental milestones but deteriorate after the age of one year (137). The leukodystrophy affects both the cerebrum and the cerebellum and can be stable or progressive. In the absence of clear-cut biochemical peroxisomal hallmarks, the differential diagnosis with other leukodystrophies is challenging. By performing sequential MRI a typical pattern was revealed for the ZSS whereby abnormalities start in the hilus of the dentate nucleus and the superior cerebellar peduncles before affecting the cerebellar white matter and the brainstem. The white matter degeneration in the forebrain appears thereafter (137). Other unique presentations were reported such as an infant with acute neurological deterioration accompanied by inflammatory demyelination in the brainstem (79). Remarkably, in the *Nestin-Pex5* mouse model lacking functional peroxisomes in neural cells, extensive inflammatory demyelination develops. This also initiated in the cerebellum and progressed to brainstem, cortex and corpus callosum mimicking the sequence in patients (13).

PEX16 mutations as a cause of cerebellar ataxia

The PEX16 protein is essential for peroxisomal membrane assembly which precedes the import of matrix proteins. In six *PEX16* mutant patients, the most pronounced neurological defects were related to cerebellar dysfunction (32). They developed spastic paraplegia and ataxia in preschool years, whereas cognitive function was unaffected. Nevertheless, besides cerebellar atrophy, widespread changes in white matter in both sub- and supratentorial regions were detected and several patients were diagnosed with a demyelinating motor and sensory neuropathy. Plasma VLCFA were mildly increased in all cases, whereas branched-chain fatty acids only in half of them.

The ring finger PEX disorders: PEX12, PEX10 and PEX2

An emerging subgroup of mild Zellweger spectrum patients present with ataxia as a primary clinical sign. Gootjes et al (55) reinvestigated a patient first diagnosed with trihydroxycholestanoic acidemia and found that PEX12 deficiency was the cause of the clinical and biochemical abnormalities. The patient presented around age 5 with mild intellectual disability, cerebellar ataxia, hypotonia, and absent reflexes. Later she developed retinopathy. Cerebellar imaging was not reported. Two patients with compound heterozygosity for PEX10 showing an analogous clinical presentation were reported by Régal et al (108). After a normal early development, they were referred to the clinic between 6 - 8 years because of worsening gait disturbances. Cerebellar abnormalities such as gait ataxia and dysarthria were confirmed by MRI, showing cerebellar atrophy. Both patients also showed an axonal motor neuropathy and posterior column dysfunction. Cognition was normal in both patients, and there was no retinopathy. Although VLCFA were not increased, a peroxisomal disorder was suspected because of increased phytanic and pristanic acid levels.

A pure cerebellar syndrome was seen in two brothers with a mutation in *PEX2* (119). Although both patients had the same mutation and showed similar clinical features, there was a striking difference in the onset of symptoms. One patient started to develop gait disturbances around 3.5 years and was diagnosed at 14 years with isolated progressive cerebellar ataxia. The other patient showed cerebellar signs such as impaired gait and dysmetria around the age of 18 years. Both patients showed pronounced cerebellar atrophy but no signs of myelin abnormalities or neuronal migration defects on MRI. Also in these patients, only pristanic acid and phytanic acid were moderately elevated whereas VLCFA levels were normal. In a third *PEX2* patient with a very similar clinical phenotype, biochemical analysis revealed a slight increase in VLCFA levels (91).

Although their numbers are still low, it is intriguing that these patients carry a mutation in *PEX12, PEX10* or *PEX2*. These peroxins act as ubiquitin ligase (E3)-like proteins and contain a RING finger domain. Mono-ubiquitination allows PEX5 to be recycled, whereas poly-ubiquitination targets PEX5 to the proteasome for degradation (50).
At present it is unclear why the mutations in these genes with a common task in the peroxisome biogenesis process give rise to the cerebellar ataxic phenotype. Metabolically, these patients share mildly increased levels of branched-chain fatty acids but minor alterations in VLCFA. Interestingly, not all mild peroxisomal biogenesis disorders cause ataxia. Indeed, the phenotype of mild PEX6 deficiency is characterized by retinopathy and deafness ((106) and personal observation), signs absent in mild PEX10 and mild PEX2 deficiency, but no cerebellar degeneration. Our current knowledge of the function of these genes as necessary for global peroxisomal function does not explain why mild defects result in gene-specific clinical syndromes. At least two possible explanations deserve further investigation. The reserve for normal peroxisomal function may be different for different PEX genes in different tissues. Another explanation would be unknown additional functions beside peroxisomal biogenesis.

CONCLUSION

It is striking that dysfunction of each of the three major peroxisomal lipid pathways gives rise to cerebellar defects. α-Oxidation impairment causes a pure degenerative process that can be halted by dietary intervention. The diversity in β-oxidation and ether lipid synthesis diseases is much wider, ranging from developmental malformations of the cerebellum and/ or brainstem to degeneration later in life. It is remarkable that, during the last decade, besides the prototypical severe peroxisomal diseases such as Zellweger syndrome, MFP2 deficiency and CCALD, a number of patients have been diagnosed with mild deficiencies. Their identification is hampered by their apparent rarity, clinical signs that are not pathognomonic such as ataxia, neuropathy and psychomotor retardation in combination with (near) normal levels of peroxisomal metabolites in blood. There is no doubt that with the increasing application of genetic techniques such as exome sequencing, many more of these patients will be identified.
The mechanisms linking metabolic abnormalities to cerebellar pathology in peroxisomal disorders remain largely unresolved. Phytanic acid levels correlate with ataxia in Refsum disease, but the precise molecular impact (in PCs) needs to be further elucidated. Furthermore, it remains unclear whether and how elevated levels of VLCFA affect the cerebellum. It is most intriguing that in recently diagnosed adolescent or adult peroxisomal patients with progressive ataxia, most peroxisomal metabolites are (near) normal, contrasting with the high concentrations necessary to cause acute neurotoxicity *in vitro*. This raises the question whether unknown metabolic factors may be at play. To sort this out, unbiased approaches should be used to unravel whether mild peroxisome dysfunction may deregulate other metabolites. With regard to the cerebellar pathologies induced by the lack of ether lipids, the recent demonstration that AKT-GSK3β signaling is impaired in the peripheral nervous system of an RCDP mouse model, might shed new light on the pathogenesis in the brain.

Cerebellar Purkinje cells are amongst the most metabolically active of all neurons, thereby making them more vulnerable for derangement of cellular homeostasis. This not only relates to peroxisomal deficiencies but also to lysosomal (8, 143), mitochondrial (23) and autophagy (1, 74) processes. In this view, it cannot be excluded that a primary peroxisomal metabolic defect impairs mitochondrial function or autophagy processes. Whereas some peroxisomal pathologies may be Purkinje cell autonomous, others are likely due to aberrations in the circuitry

innervating these pivotal cells. This can be addressed by creating mouse models with PC selective inactivation of peroxisomal proteins. It remains to be elucidated whether neurons in other brain areas develop similar pathologies at later time points as a consequence of peroxisome dysfunction.

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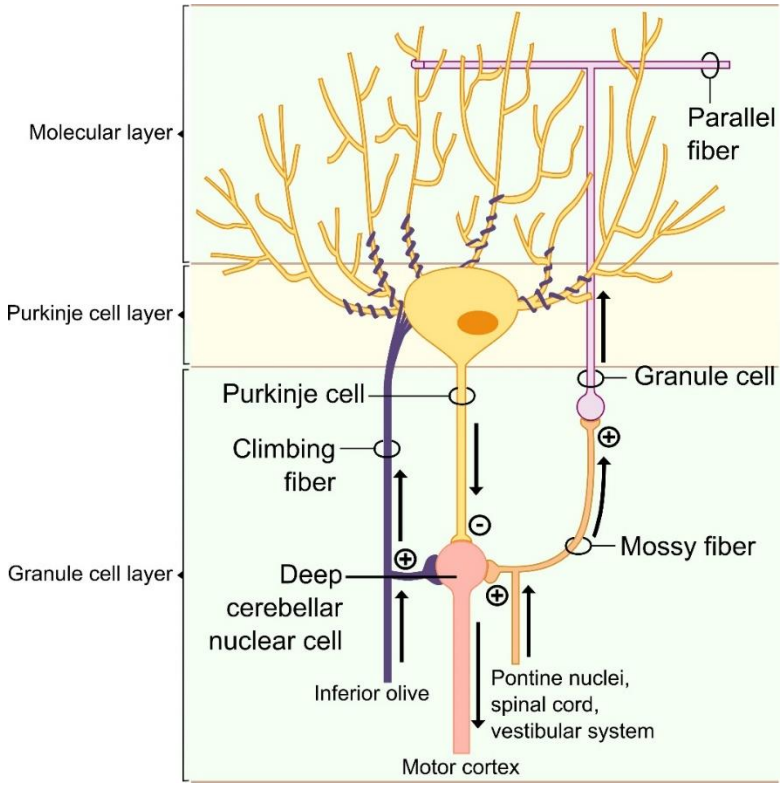
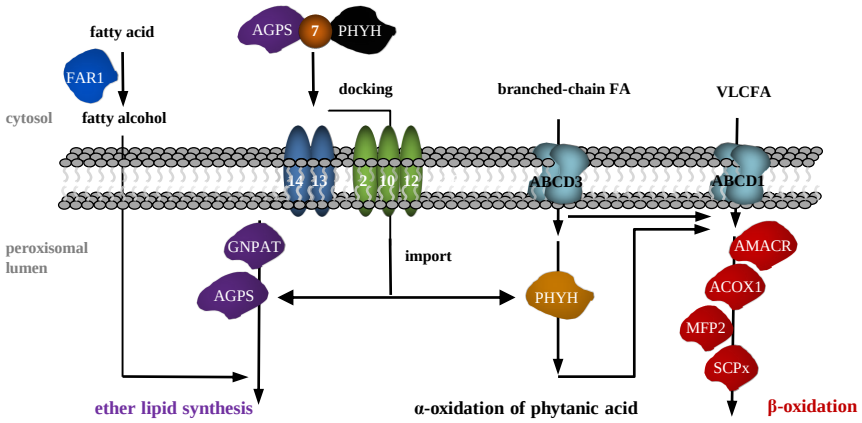


Figure 1: Cerebellar architecture. The cerebellar cortex harbors five cells types. The Purkinje cells are the most obtrusive, appearing as an ordered monolayer located in between the inner granular and the outer molecular cell layer. By providing the only projections to the deep cerebellar and vestibular nuclei, these cells are the sole cerebellar output neurons. Mossy fibers arising from the pontine nuclei, spinal cord and vestibular system project into the granule cell layer to establish synaptic contacts on the granule cells. In turn, parallel fibers arising from granule cells project into the molecular layer to make excitatory synapses on the elaborated dendritic three of the Purkinje cell. Climbing fibers arising from the inferior olive provide the second Purkinje cell excitatory innervation. The deep cerebellar nuclei are stimulated by mossy fibers and efferents originating from the inferior olive, while Purkinje cells provide inhibitory input.



PATIENTS

Ether lipid synthesis

Mutations in *PEX7*, *GNPAT* and *AGPS*: RCDP type 1, 2 or 3

- Cerebellar atrophy and heterotaxias
- Dysplastic olives

FAR1 deficiency

- Cerebellar atrophy

α-oxidation

Mutations in *PHYH*: Refsum disease

- Cerebellar atrophy
- Degeneration of dentate and inferior olivary nuclei

β-oxidation

AMACR deficiency

- Degeneration of afferent and efferent cerebellar pathways

Mutations in *ABCD1*: X-ALD

X-ALD with changes in the dentate nuclei and cerebellar white matter

AMN with cerebellar atrophy or cerebellar white matter changes

Atypical X-ALD variants:

- White matter lesions in cerebellum and/or brainstem
- Dentate nucleus pathology
- Atrophy of the cerebellum and brainstem

Acox1 deficiency

Severe

- Demyelination of cerebellar - and brainstem tracts

Mild

- Atrophy of the cerebellum and brainstem

MFP2 deficiency

Severe

- Ectopic or degenerating PCs
- Defects in ganule cell migration and maturation
- Gliosis
- Cerebellar and brainstem demyelination
- Malformations of inferior olivary and dentate nuclei
- Cerebellar atrophy and hypoplasia

Mild

- Cerebellar demyelination
- Cerebellar atrophy in childhood, adolescence or adulthood

SCPx deficiency

- ND

ABCD3 deficiency

- ND

MOUSE MODELS

Ether lipid synthesis

Elimination *GNPAT*: RCDP type 2 model

- Delayed cell migration
- Disturbed foliation
- PC abnormalities
- Cerebellar dysmyelination
- Altered CF and PF innervation
- Cerebellar atrophy

α-oxidation

Elimination of *PHYH*: Refsum disease model

- Focal to severe PC loss *

β-oxidation

Elimination of *Abcd1/Abcd2*: X-ALD

- PC atrophy and death

Acox1 deficiency

- ND

MFP2 deficiency

(Nestin-)Mfp2$^{-/-}$ mice

- Delay in cerebellar foliation (*Mfp2*$^{-/-}$)
- Cerebellar micro- and astrogliosis
- Axonal swellings
- Cerebellar demyelination
- Cerebellar atrophy with PC degeneration (*Nestin-Mfp2*$^{-/-}$)

SCPx deficiency

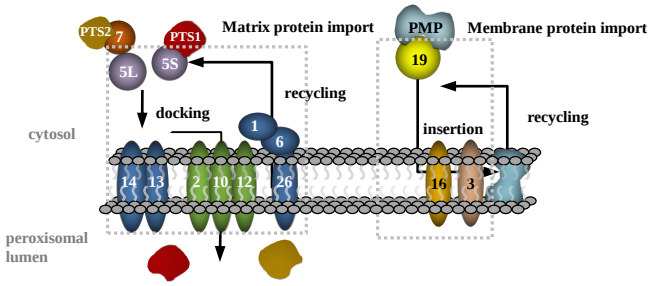
- ND

ABCD3 deficiency

- ND

Figure 2: Schematic overview of cerebellar and brainstem pathologies in peroxisomal single enzyme and transporter defects in men and mice. Defects in the three major lipid metabolic pathways of peroxisomes in men and mice cause developmental or degenerative pathologies. Ataxia is a common phenotype. Figure adapted from (Waterham et al. 2012). ND = not documented; * after phytol treatment

Figure 3: Peroxisomal biogenesis defects



Patients

Mutations in PEX genes lead to Zellweger syndrome spectrum (ZSS)

Severe

- Hampered cerebellar neuronal migration and heterotopic Purkinje cells
- Malformations of the olivary and dentate nuclei

Mild

- Cerebellar atrophy
- Late-onset cerebellar ataxia
- Peripheral neuropathy

Mouse models

Elimination of PEX2, PEX5 and PEX13 genes:

Zellweger syndrome phenotype

- Disturbed cerebellar foliation
- Impaired granule cell migration
- PC heterotopias and altered morphology
- Abnormal CF innervation

Figure 3: Schematic overview of cerebellar and brainstem pathologies in peroxisomal biogenesis defects (PBD) in men and mice. PBD are caused by mutations involved in peroxisomal matrix protein import and peroxisomal membrane protein (PMP) import. Peroxisomal matrix proteins contain a C-terminal (PTS1) or a N-terminal (PTS2) peroxisomal targeting signal. PTS1 matix proteins are recognized by a shorter variant of the peroxisomal import receptor PEX5 (5S). The PTS1/PEX5 complex binds to the docking complex at the peroxisomal membrane which leads to import of the protein in the peroxisomal lumen. PTS2 proteins are imported in a similar way, but are first recognized by PEX7 that binds to the longer variant of PEX5. Peroxisomal membrane proteins are incorporated via a mechanism involving PEX19, PEX16 and PEX3.